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PHYCOPERIPHYTON (ALGAE) AS INDICATORS OF WATER QUALITY[1]

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COLLINS, G. B. & WEBER, C. I. 1978. Phycoperiphyton (Algae) as indicators of water quality. *Trans. Amer. Micros. Soc.*, 97: 36–43. The attached algal community is an important component of aquatic ecosystems and has unique attributes for water quality studies which stem not only from its habit but also from its species composition. Several sampling methods have been developed that provide useful data on properties of this phycoperiphyton community. Involved are biomass measurements, pigment composition, productivity, community structures and species composition. These features are reviewed here. Routine measurement of the basic properties of periphyton in surface water monitoring programs is highly recommended. To help handle the large amounts of data on the phycoperiphyton (and other communities of aquatic organisms) that should result from these studies, the EPA is developing a general computerized biological data management system.

The term periphyton is of Russian origin (Behning, 1928) and first referred only to organisms growing on objects placed in water by man. It gradually acquired a broader meaning (Young, 1945); and it is now generally employed to include all attached microorganisms growing on natural or artificial substrates (Amer. Public Health Assoc., 1971), a usage similar in meaning to the German term *Aufwuchs*. Foerster & Schlichting (1965) introduced the term *phyco*-periphyton to describe the algal component. The attached nature of the periphyton makes it particularly useful in streams, where it may serve as a reliable index of the water quality at a given sampling point. Metabolic activities are the first to be affected by changes in water quality, whereas community structure responds more slowly as populations readjust to new conditions in terms of their environmental requirements and pollution tolerance.

The most recent extensive reviews of the literature on the subject are those of Cooke (1956), on use of artificial substrates; Lund & Talling (1957), on algal methodology in general; Sládečková (1962), specifically on methodology of the periphyton; and Wetzel (1964), who placed special emphasis on measurement of biomass and production rates. Extensive bibliographies on the periphyton are published annually by the North American Benthological Society (Weber, 1966–1973; Collins, 1974, 1975).

SAMPLING METHODS

Although quantitative sampling from natural substrates is difficult, samples containing members of most of the taxa occurring in a given environment can be obtained by scraping techniques. The problem of obtaining uniform samples led to the early use of artificial substrates. Hentschel (1916) was the first to describe the use of glass slides as artificial substrates for quantitative and qualitative studies of periphyton communities. Glass slides have become the

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most widely used material and, despite some bias, provide the advantage of uniformity, so important in quality control.

The most widely employed phycoperiphyton samplers in the United States are floating devices which usually resemble the Catherwood Diatometer described by Hohn (1954). There are numerous modifications of this design. The sampler widely employed in the Federal water pollution control program has a Plexiglas frame and Styrofoam floats (Weber, 1966, 1973a); it differs from the Catherwood Diatometer in that it lacks the V-shaped shield forward of the slide rack. Sampling equipment may now be obtained from commercial sources, but in the past it was generally custom-made.

Criteria to be considered in the selection of samplers include the following: (1) It should have only one stable position (upright); (2) the float must be insoluble in gasoline and other hydrocarbons which may be spilled on the water; (3) metal parts must be noncorrosive; (4) the slides should rest on edge, with their long axes parallel to the current; and (5) the upper edge of the slides should be held 20–30 mm below the surface.

Although the use of artificial substrates is often desirable, there are many types of investigations where the natural substrate must be used. The newer SCUBA techniques may provide the only way to sample in deep water or where sampling techniques require personnel to remain under water for prolonged periods of time (Gale & Thompson, 1974).

The length of the exposure period is a very critical factor. The most important consideration is that a sufficient amount of material be collected to carry out the desired analyses without exposing the substrate so long that part of the material sloughs off and the integrity of the sample is lost. The rate of colonization is largely dependent on the temperature, trophic level, and velocity of the water. As a rule, for studies of both algal species diversity and biomass measurements, an exposure period of 2–4 weeks is recommended in midsummer in eutrophic waters of low velocity. If diatoms are the only organisms to be studied, a two-week period is usually adequate. However, if all the algae in the community are of interest, a longer exposure period may be necessary.

Wetzel (1966) pointed out that the productivity of periphyton collected on floating samplers placed in the deep water areas of lakes and in the middle of rivers may bear little relationship to natural periphyton which occurs in the littoral areas. However, the objectives of the study must be considered when selecting the sampling technique. If the primary objective is to monitor changes in trophic status or pollution levels over an extended period of time, as many variables as possible should be eliminated. Artificial substrates provide more precise data than natural ones and would be the method of choice. Patrick et al. (1954) and others have pointed out that the diatoms collected on glass slides exposed for two weeks are essentially the same as those of the natural community. Hohn & Hellerman (1963) reported similar results, but also showed that, in cold water, samples from Styrofoam substrates were more like the natural community than those from glass slides.

Artificial substrates attached to fixed objects, such as dam faces or pilings, or simply resting on the bottom may not provide samples of adequate reproducibility for use in monitoring activities because the depth of exposure and turbidity may change considerably from time to time. To determine the effect of the depth of substrate placement on the properties of the community, we exposed glass slides at 10 cm intervals in the upper meter of a reservoir.

Large differences were found in the species composition with increasing depth, indicating the confounding effect of this factor in evaluating the relationship between the properties of the community and water quality. To avoid this problem, the substrates should be exposed just below the surface of the water, as described above.

Biomass Measurements

Total periphyton biomass and phycoperiphyton biomass are related to water quality and are commonly measured in terms of ash-free weight (AFW) (dry weight organic matter) and/or chlorophyll *a*.

Ash-free Weight

AFW is obtained by heating the sample at a temperature of 105°C to constant weight, and igniting in a furnace at 500°C for one hour (APHA, 1976; Weber, 1973b). A value similar to AFW is obtained by the infra-red measurement of CO_2 liberated during wet combustion, as described by Menzel & Vaccaro (1964).

Chlorophyll

Chlorophyll *a* is widely used to estimate phycoperiphyton biomass and to characterize the periphyton community structure (Weber, 1973a, b). However, errors may arise in measuring chlorophyll because of its instability. The extracted substance is very sensitive to light and will degrade at a rate of ca. 3% per hour at normal laboratory light intensities; therefore, extreme care should be taken to work in subdued light. Ninety percent aqueous acetone is the most advantageous solvent in which to make measurements, but it does not quantitatively extract chlorophyll from some organisms (e.g., the coccoid green algae).

Samples must be routinely mascerated to ensure complete extraction. The chlorophyll content may be determined fluorometrically or spectrophotometrically (Weber, 1973b). For accurate work, the spectrophotometer employed must have a resolution of at least 2 nm. The use of inexpensive instrumentation with a nominal resolution as high as 10–20 nm will result in an underestimation of the chlorophyll *a* values by as much as 40%. The presence of chlorophyll degradation products, such as pheophytin *a*, in dead or dying cells beneath the surface of the periphyton mat may cause significant errors in the estimates. It is important, therefore, to routinely determine the pheophytin *a* content of pigment extracts by measuring the optical density or fluorescence before and after acidification.

AFW:Chlorophyll Relationship

Chlorophyll data may be used to estimate algal biomass. In a recent review, Weber (1973a)—with reference to algal cultures—listed AFW:chlorophyll *a* ratios ranging from 40 to 96. McIntire & Phinney (1965) reported AFW:chlorophyll *a* ratios from 50 to 250 for periphyton in laboratory streams; these workers discussed some of the conditions responsible for the variation, such as species composition and light intensity. Holm-Hansen (1969) used a factor of 100 to convert chlorophyll *a* to biomass in marine plankton. Until more data are available, we suggest that a factor of 67 is most appropriate to use in converting chlorophyll *a* to phycoperiphyton biomass (Weber, 1973a).

DNA and ATP

A fluorometric method for the measurement of the DNA content in marine plankton was described by Holm-Hansen et al. (1968), but it has not been used to estimate periphyton biomass. The same is true of the luciferin-luciferase method of measuring ATP in marine plankton (see Holm-Hansen & Booth, 1966), which could also be applied to periphyton. Work done in our laboratory indicates that ATP can be successfully measured if the sample is extracted in the field or quickly frozen for later extraction in the laboratory. A simple method employing nitric acid, has been used by Chappelle et al. (1975) with bacteria; it may be considered applicable to the periphyton as well.

In an extensive review of the ATP:biomass relationship in microorganisms, Weber (1973a) suggested the use of a factor of 400 to convert ATP to viable biomass. Holm-Hansen (1969) used an ATP to biomass conversion factor of 250 in studies of marine plankton and, more recently, (Holm-Hansen, 1973) reported a biomass:ATP ratio of 286 in algal cultures and 265 in marine plankton.

Productivity

Productivity can be measured in situ or in vitro. Results in both cases, however, are subject to large errors: in the former, only crude estimates can be made of gas diffusion rates and other factors; and in the latter, the community must be removed from the flowing water environment and placed in sealed bottles. Methods of measuring productivity are discussed by King & Ball (1966), Sládeček & Sládečková (1964), and Wetzel (1964, 1966), and in Standard Methods, 14th edition (APHA, 1976).

Community Structure

Community structure is closely related to water quality and is commonly expressed in terms of the composition of the chlorophylls, the AFW:chlorophyll *a* ratio, or the species composition.

Chlorophyll Composition

All algae contain ca. 1–2% chlorophyll *a* on a dry weight organic matter (AFW) basis. However, the major taxonomic groups of these organisms differ significantly in their content of chlorophyll *b* and *c*. For example, blue-green "algae" contain chlorophyll *a* only; green algae contain chlorophyll *a* and *b* in a ratio of 2:1; and the diatoms contain only chlorophyll *a* and *c*, at ratios ranging from 7:1 to 1:1. Thus, useful information on the taxonomic composition of the phycoperiphyton can be obtained by carrying out a spectrophotometric analysis of chlorophyll extracts, using the UNESCO (1966) trichromatic equations for the simultaneous determination of chlorophyll *a*, *b*, and *c* in the samples.

AFW:Chlorophyll Ratio

The AFW:chlorophyll *a* ratio of the periphyton is very useful in determining the relationship between the biomass of the autotrophic and heterotrophic elements of the community (Weber, 1973a). The periphyton of surface waters containing negligible amounts of degradable organic matter and other pollutants usually consists largely of algae and has an AFW:chlorophyll *a* ratio in the range of 50–100. However, if degradable organic matter is discharged into the water, the biomass of the nonchlorophyllous (heterotrophic) microorganisms

will increase more rapidly than that of the algae, causing an increase in the ratio. In samples collected from surface waters receiving large amounts of domestic waste, this ratio, called the Autotrophic Index, will have values in the range of 400–1,000 or higher. Use of this Index has been included in the Model State Water Monitoring Program (USEPA, 1975) and the Basic Water Monitoring Program developed by the Agency (USEPA, 1977).

Species Composition

Data on species composition are used to characterize water quality with respect to individual indicator species, species associations, species richness, and relative abundance of species (including mathematical analyses and diversity indices).

If considerable information is available on the environmental requirements and pollution tolerance of a given organism, it may serve as a useful indicator species. This concept was first developed by Kolkwitz & Marsson (1902, 1908, 1909), who used many species of the phycoperiphyton in their "saprobic" system of water quality classification. The system was further refined in subsequent reports by Kolkwitz (1935, 1950), and it has been used extensively in Europe. Fjerdingstad (1950, 1963, 1964, 1965) described what he termed "a new saprobic system, based on communities of phyto-microorganisms (synecology)." He found that evaluations of water quality were less conclusive when based on individual indicator species than when based on the species composition of the entire community. He recognized that the taxonomy of many of the phycoperiphyton is difficult and that to use his scheme the "saprobic valency" of each organism must be known. Butcher (1932, 1940, 1947, 1948) and Cholnoky (1958) have also contributed extensively to the literature on the taxonomic composition of the phycoperiphyton under the influence of different types of pollution. The most recent and most extensive review of the saprobic system is that of Sládeček (1973).

It has been recognized that the diatoms constitute a major portion of the phycoperiphyton and, because they are more easily identified to the species level than many other algae and differ in their environmental requirements, that they are especially useful as indicators of water quality. Excellent reviews of their use in such a way have been published by Patrick (1971, 1973).

Much of the data on the ecology of the diatoms is widely scattered in the literature and largely inaccessible. To enhance the usefulness of these algae as water quality indicators a project was funded by our program to compile and summarize ecological data on the common fresh-water species. The resulting report (Lowe, 1974), following the format of the "Microalgal Spectral Analysis" developed earlier by Abbott & van Landingham (1972), contains information on the following parameters: pH, nutrients, salinity, organic pollution tolerance (saprobity), current, general habitat, specific habitat, seasonal distribution, temperature, and geographical distribution. A set of precisely defined terms was included to encourage standardization of the nomenclature.

The mathematical analysis of phycoperiphyton community structure was pioneered by Patrick et al. (1954), who employed the truncated normal curve to evaluate the effects of water quality on diatom species diversity. Unfortunately, this method is dependent on very large (detailed) counts of the community and thus has not been widely used. Successful development of an automated diatom identification system, such as the one under development at the Virginia Polytechnic Institute and State University (Cairns et al., 1973), would greatly reduce the analysis time and provide a much larger data base

on which to model and evaluate the relationship between water quality and (diatom) community structure. Other more recent mathematical analyses include species diversity indices and cluster analysis (Margalef, 1957; Wilhm, 1970).

Data Management

The extensive use of the periphyton (and other aquatic organisms) in water quality studies will result in the accumulation of large amounts of data on standing crop, community structure, and metabolism, and will require the availability of a computerized biological data-handling system. In response to this need, we are developing a system called Bio-Storet (General Electric, 1973) which is scheduled to be implemented in the very near future.

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